Remarks

Applicants thank the Examiner for withdrawing the rejections made in the previous Office Action and for the allowance of claims 22, 31, 33-35, 40, and 44.

The Amendments

Claim 22 has been amended to recite "an isolated" polynucleotide. Independent claims 31 and 35 have been amended to recite "an isolated" recombinant human fibroblast growth factor receptor vector. Isolated polynucleotides and vectors are disclosed, for example, in Example 2.

Claim 37 has been amended to recite conditions that permit hybridization "to both oligonucleotide probes." This recitation is supported by the last phrase of claim 37.

The amendments add no new matter.

The Objection to Claims 22, 31, 33-35, 37, 40, and 44

Claims 22, 31, 33-35, 37, 40, and 44 are objected to because they recite a composition consisting essentially of "only one item" and "a composition implies more than one substance." Office Action at page 2, item 3. The Office Action cites no authority for this objection, which Applicants respectfully traverse.

Nonetheless, to advance prosecution, independent claim 22 has been amended to recite "an isolated" polynucleotide. Independent claims 31 and 35 have been amended to recite "an isolated" recombinant human fibroblast growth factor receptor vector. Corresponding amendments have been made to dependent claims 33 and 34. Claims 37, 40, and 44 do not recite a composition and appear to have been erroneously included in the objection.

The Rejection of Claim 37 Under 35 U.S.C. § 112, second paragraph

Claim 37 stands rejected under 35 U.S.C. § 112, second paragraph, as indefinite. Applicants respectfully traverse the rejection.

The second paragraph of 35 U.S.C. § 112 states that:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

It is well settled that a claim must "reasonably apprise those skilled in the art both of the utilization and scope of the invention." *Georgia-Pacific Corp. v. United States Plywood Corp.*, 258 F.2d 124, 134-38, 118 U.S.P.Q. 122, 130 (2d Cir. 1958), *cert. denied*, 358 U.S. 884 (1958). Claim 37 as amended meets this standard.

Claim 37 recites a method of isolating a polynucleotide having a sequence encoding a human fibroblast growth factor receptor (hFGFr) comprising the amino acid sequence shown in SEQ ID NO:1. The method comprises providing two oligonucleotide probes and a cDNA library of candidates. The cDNA library is contacted with the probes under conditions that permit hybridization to both oligonucleotide probes and identifying and isolating the candidate that hybridizes to both oligonucleotide probes.

The Office Action asserts that the recitation of the hybridization conditions is vague and indefinite because "it is not known what these conditions are." Office Action at page 2, last paragraph. The Office Action "require[s] that Applicants amend the claim[] to recite the exact hybridization conditions." Sentence bridging pages 2 and 3. "Exact" hybridization conditions, however, are not required to reasonably apprise those skilled in the art of the utilization and scope of claim 37.

Claim 37 as amended explicitly states that the hybridization conditions "permit hybridization to both oligonucleotide probes." Hybridization conditions that permitted hybridization of oligonucleotide probes to cDNA libraries for purposes of isolating a candidate polynucleotide were well known in the art when this application was filed. For example, the second edition of Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (referred to at page 15, lines 16-17 of the specification), states that

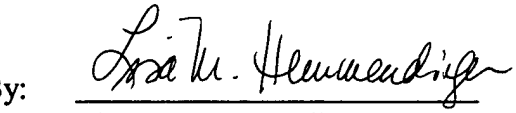
> as a result of more than 20 years of work, the theoretical basis of nucleic acid hybridization is well-understood. This has led to the development of a large number of different techniques that can accommodate nucleic acid probes of very different lengths and specificities.

Page 8.46. A section of Sambrook *et al.* that describes methods of screening to identify cDNA clones of interest is attached. In view of the "large number of different techniques" known for this purpose, as well as the familiarity of those skilled in the art with nucleic acid hybridization, those skilled in the art would readily be able to identify and use a variety of hybridization conditions to isolate the recited polynucleotide. Thus, amendment of claim 37 to recite "exact" hybridization conditions is unnecessary to render claim 37 definite.

Applicants respectfully request withdrawal of the rejection.

Respectfully submitted,
BANNER & WITCOFF, LTD.

Date: January 27, 2004

By: 

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Attachment 1

Molecular Cloning

A LABORATORY MANUAL

SECOND EDITION

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GENETICS INSTITUTE

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HARVARD UNIVERSITY

1890–1990 CSH CENTENNIAL

Cold Spring Harbor Laboratory Press
1989

IDENTIFICATION OF cDNA CLONES OF INTEREST

Methods of Screening

There are three methods to screen cDNA libraries for clones of interest:

- Nucleic acid hybridization
- Immunological detection of specific antigens
- Sib selection either by hybrid selection and translation of mRNA or by production of biologically active molecules

Most cloning projects today are aimed at isolating cDNAs corresponding to rare mRNAs and therefore require screening of large numbers of recombinant clones. This can be carried out effectively with only two types of reagents: antibodies and nucleic acid probes. In those rare instances when both types of reagents are available, nucleic acid probes are preferred because they can be used under a variety of different stringencies that minimize the chance of undesirable cross-reactions. Furthermore, nucleic acid probes will detect all clones that contain cDNA sequences, whereas antibodies will react only with a subset of these clones (in some cases one in six at best) in which the cDNA has been inserted into the vector in the correct reading frame and orientation. cDNA libraries that are to be screened by antibodies therefore need to be larger (by a factor of at least 6) than those that are to be screened by nucleic acid probes. Consequently, when using antibody probes to search for a cDNA clone corresponding to a mammalian mRNA present at the level of 1 molecule/cell or less, it is desirable to construct cDNA expression libraries that contain in excess of 10^7 members. This is not easy, especially when the amounts of mRNA are limited. Furthermore, screening a library of this size is expensive and laborious, and it becomes worthwhile to explore methods to enrich the mRNA (or cDNA derived from it) for the sequences of interest (see pages 8.6–8.10).

NUCLEIC ACID HYBRIDIZATION

This is the most commonly used and reliable method of screening cDNA libraries for clones of interest. None of the other methods displays such an abundance of attractive features. Screening by nucleic acid hybridization allows extremely large numbers of clones to be analyzed simultaneously and rapidly, does not require that the cDNA clones be full-length, and does not require that an antigenically or biologically active product be synthesized in the host cell. Furthermore, as a result of more than 20 years of work, the theoretical basis of nucleic acid hybridization is well-understood. This has led to the development of a large number of different techniques that can accommodate nucleic acid probes of very different lengths and specificities. Details of the methods for the preparation and use of these probes are presented in Chapters 10 and 11.

Homologous probes

Homologous probes contain at least part of the exact nucleic acid sequence of the desired cDNA clone. They are used in a variety of circumstances, for

example, when a partial clone of an existing cDNA is used to isolate a full-length clone from a cDNA library. Usually, a fragment derived from one end or the other of the existing clone is isolated, radiolabeled in vitro, and used to probe a library. Hybridization with homologous probes is always carried out under stringent conditions.

Partially homologous probes

Partially homologous probes are used to detect cDNA clones that are related, but not identical, to the probe sequences. If neither antibody nor nucleic acid probes are available, a number of alternative strategies can be considered. For example, if the same gene has already been cloned from another species or if a related gene has been cloned from the same species, it would be worthwhile carrying out a series of trial experiments to determine whether there is sufficient conservation of nucleic acid sequence to allow the screening of a cDNA library by hybridization. This is most easily accomplished by performing a series of Southern and northern hybridizations at different stringencies. For example, a large batch (50 μg) of genomic DNA is cleaved with a restriction enzyme that cleaves the probe sequence at one or two well-separated sites. It is a good idea to digest an equal amount of genomic DNA of the original species for use as a positive control. Aliquots (5–10 μg) of the digests are then applied to adjacent slots of a 0.8% agarose gel, electrophoresis is carried out, and the fragments are then transferred to a nitrocellulose filter as described in Chapter 9, pages 9.34–9.41. The filter is cut into strips, each of which is hybridized under different conditions to identical amounts of radioactive probe. For aqueous hybridization, the ionic strength of the solution is kept constant (usually 1 M Na^+) while the temperature of annealing is progressively lowered (from 68°C to 42°C). The strips are then washed extensively at the temperature of hybridization with a solution containing 2 × SSC, 0.5% SDS. When hybridization is carried out in solvents containing formamide, the temperature and ionic strength are usually kept constant (42°C and 6 × SSC [or 6 × SSPE], respectively) while the amount of formamide in the annealing buffer is progressively lowered from 50% to 0%. The strips are then washed extensively at 50°C in 6 × SSC, 0.5% SDS. A similar series of hybridizations can be carried out with mRNA preparations that have been fractionated by electrophoresis and transferred to a solid support. In both cases, the aim is to establish conditions that will allow the previously cloned gene to be used as a probe for the cDNA of interest, without undue interference from background hybridization.

Total cDNA probes

Total cDNA probes are prepared by uniform incorporation of radiolabeled nucleotides with reverse transcriptase or end-labeling of total or fractionated poly(A)$^+$ mRNA. They can be used to screen libraries of cDNA for specific clones *if* the cDNA clones of interest correspond to mRNA species present in the initial population at a frequency of at least 1 in 200 (see Gergen et al. 1979; Dworkin and Dawid 1980). It is not possible to detect cDNA clones homologous to species that are represented rarely in the mRNA preparation.

Subtracted cDNA probes

Subtracted cDNA probes are often used to probe cDNA libraries for clones that correspond to mRNAs that are differentially regulated. A cDNA probe prepared from one type of mRNA is depleted of sequences that are present in a second type of mRNA by subtractive hybridization (Timberlake 1980; Zimmerman et al. 1980). Typically, the cDNA is hybridized two or three times in succession to a 20-fold excess of the second mRNA, and the cDNA:mRNA hybrids are removed by chromatography on hydroxyapatite. The unhybridized cDNA is then annealed to a 100-fold excess of the mRNA preparation from which it was originally synthesized, and the resulting cDNA:mRNA hybrids are this time recovered by chromatography on hydroxyapatite. After the mRNA is removed by alkaline hydrolysis, the cDNA, which is highly enriched for sequences specific to the original mRNA, is used to probe a cDNA library for clones homologous to these sequences.

Subtracted cDNA probes are particularly valuable when there are very few differences between the two starting mRNA preparations, i.e., when most species of mRNA are represented equally in the two preparations and a small proportion (<2%) of the mRNAs are not present at all in one preparation. cDNAs that have been cloned using subtracted cDNA probes include the murine J immunoglobulin chain (Mather et al. 1981) and the murine T-cell receptor (Hedrick et al. 1984).

A slightly different approach is used when two preparations of mRNA share sequences that are present at different concentrations. Examples of such sib pairs might be mRNAs extracted from control cells and cells that have been exposed to heat shock, drugs, or hormones. cDNAs corresponding to mRNAs whose expression is altered by such treatments can often be detected by *differential hybridization*. ^{32}P-labeled first-strand cDNAs are synthesized in vitro using both mRNAs as templates. Most of the cDNA sequences correspond to mRNAs whose concentrations are not appreciably changed by the treatment to which the cells were exposed. However, a minority of the cDNAs will be copied from mRNAs whose concentrations are significantly increased or decreased. The two cDNA probes are then used to screen replicas of a cDNA library constructed from mRNA extracted from control cells (when searching for mRNAs that are repressed) or treated cells (when searching for mRNAs that are induced). The clones that hybridize preferentially to one of the cDNA probes are chosen for further analysis. Among the many examples of genes cloned in this way are the galactose-inducible genes of yeast (St. John and Davis 1979), human fibroblast interferon (Taniguchi et al. 1980), the glucose-regulated proteins of mammalian cells (Lee et al. 1981), growth-related proteins (Foster et al. 1982; Cochran et al. 1983; Linzer and Nathans 1983), differentiation-specific proteins (Spiegelman et al. 1983), and a variety of heat-shock proteins and stress proteins (see, e.g., Mason et al. 1986). The procedure has also been used to identify cDNA clones of developmentally regulated mRNAs from organisms of many different species including *Xenopus* (Williams and Lloyd 1979; Weeks et al. 1985), *Dictyostelium* (Rowekamp and Firtel 1980; Mehdy et al. 1983), sea urchins (Lasky et al. 1980), and mice (Gorman et al. 1985).

Synthetic oligonucleotide probes

Synthetic oligonucleotide probes are tracts of dNTPs of defined sequence that have been synthesized in vitro. The sequence of these probes is deduced, using the genetic code, from short regions of the known amino acid sequence of the protein of interest. Because of the degeneracy of the genetic code, it is very unlikely that a given sequence of amino acids will be specified by a predictable single oligonucleotide of defined sequence. Instead, in the vast majority of cases, the same sequence of amino acids can be specified by many different oligonucleotides. There is no way to know with certainty which of these oligonucleotides is actually used in the gene of interest. Three solutions have been found to this problem:

1. A family of oligonucleotides can be synthesized containing all possible sequences that can code for a given sequence of amino acids. The number of members in this family depends on the degree of degeneracy of the codons for the particular amino acids. However, since all possible oligonucleotide sequences are represented, at least one of the members will match perfectly with the cDNA clone of interest. To keep the size of each family within manageable proportions, short oligonucleotides (14–17 nucleotides) are generally used—the minimum size that is practical for hybridization. Often, more than one family of oligonucleotides is synthesized based on separate sequences of amino acids.
2. A longer (40–60-base) oligonucleotide of unique sequence can be synthesized using the most commonly used codon for each amino acid. (Avoid using the dinucleotide CpG, since it is underrepresented in most eukaryotic DNAs.) Almost certainly, this oligonucleotide will not match exactly the sequence in the cDNA, but it will fit well enough to be detected by hybridization under nonstringent conditions.
3. An oligonucleotide can be synthesized that contains a base such as inosine at positions of high potential degeneracy. Inosine can pair with all four conventional bases without seriously compromising the stability of the resulting hybrid. It is therefore possible to generate families of longer oligonucleotides that are reduced in number and yet are capable of hybridizing to virtually all cDNA clones that are likely to code for the protein of interest.

Finally, if the protein sequence available is from the amino terminus of the protein, the cDNA library that is to be screened must be of high quality to ensure that most of the 5′ terminus of the mRNA is represented. For a detailed discussion of synthetic oligonucleotide probes, see Chapter 11.

IMMUNOLOGICAL DETECTION OF SPECIFIC ANTIGENS

cDNA libraries constructed in expression vectors such as λgt11, λgt18–23, λZAP, and λORF8 can be screened with antibody directed against the protein of interest (see Chapter 12 for experimental details). Nitrocellulose filters imprinted with the detritus of bacterial lysis are soaked in a solution